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## EFFECTS OF POTATO BEETLE HEMOLYMPH INJECTIONS ON PHYSIOLOGICAL RESPONSES IN THE ALBINO RAT<sup>1</sup>

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### ABSTRACT

The hemolymph of both the potato beetle, *Leptinotarsa decemlineata*, and the false potato beetle, *Leptinotarsa juncta*, injected intraperitoneally into white rats produced deviant physiological responses. Monitoring showed a decrease in injected rats' temperatures. Injected rats showed an increase in serum glucose and serum urea. An increase in the serum-enzyme activity of serum glutamic pyruvic transaminase, serum glutamic oxalacetic transaminase, and lactic dehydrogenase also occurred. Fluctuations in serum ions showed a decrease in sodium concentration, but an increase in levels of potassium, calcium, and phosphorus. Beetle-hemolymph injections appeared to cause a change in the rats' hemic tissue integrity. Perturbation of the hemic integrity resulted in a state of shock which resembled anaphylactic shock.

### INTRODUCTION

Recently it has been demonstrated that the hemolymph of the Colorado "true" potato beetle, *Leptinotarsa decemlineata*, is toxic to mice (Hsiao and Fraenkel, 1969). Gross observations indicated that beetle hemolymph also had a generalized toxic effect on rats. It was of interest therefore to examine further the toxic effects of potato-beetle hemolymph on some of the rat's physiological responses. Holtzman white rats, rather than mice, were used in order to obtain sufficient blood with which measurements could be made. The physiological parameters monitored on the rats were body temperature, respiratory rate, heart rate, hematocrit, blood urea, serum glucose, serum transaminase, lactic acid enzyme activity, and serum electrolyte levels. The hemolymph of the false potato beetle, *Leptinotarsa juncta*, was likewise investigated, because this beetle also utilizes a toxic plant for its food source. The acute toxicity of the hemolymphs of these two beetles and their effects on rats' physiological responses are described here.

### MATERIALS AND METHODS

Field-collected true potato beetles, *Leptinotarsa decemlineata*, and field-collected false potato beetles, *Leptinotarsa juncta*, were used to establish cultures in the laboratory. Cultures of the true potato beetle were maintained in the laboratory on potato leaves. Cultures of the false potato beetle were reared on leaves of horsenettle, *Solanum carolinense*.

Possible toxic effects of hemolymph from both kinds of beetles were tested by intraperitoneal injection into Holtzman white rats. Rats were used in groups of 10—10 experimental and 10 control animals—in most experiments. Each sample contained 50 $\mu$ l of hemolymph taken from true adults, true fourth-instar larvae, false adults, and false fourth-instar larvae. Control hemolymph was obtained from field-collected grasshoppers of the genus *Melanopus*, taken from the same

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area as the beetles. These grasshoppers were used as controls because of their fairly large size, which facilitated obtaining hemolymph from them.

Body temperature, respiratory rate, and heart rate of rats were monitored with a Physiograph Six, using a Small Animal Study Unit (Narco Bio-Systems, Inc., Houston, Texas). Microhematocrit determinations were made using heparinized capillary tubes. Urea was determined from Somogyi filtrate of hemolyzed blood samples by the colorimetric technique of Archibald (1945). Serum glucose was measured by an enzymatic colorimetric procedure using the enzymes glucose oxidase and peroxidase according to the method of Raabo and Terkildsen (1960).

Determinations of glutamic oxalacetic transaminase (SGO-T) and glutamic pyruvic transaminase (SGP-T) were made according to the colorimetric methods of Reitman and Frankel (1957). Serum lactic dehydrogenase (LDH) was determined according to Cabaud and Wroblewski (1958). All enzyme activities are listed in international units.

Determinations of sodium and potassium were made by flame-photometric procedures of Holiday and Preedy (1953). Calcium was determined by the procedures of Bett and Fraser (1959). Inorganic phosphorus was quantitated by the colorimetric procedure of Taussky and Shore (1953).

### RESULTS

Within 30 minutes after injection of 50  $\mu$ l of hemolymph from both true potato beetle adult and larva and from false potato beetle adult and larva, a decrease in body temperature of the injected rat occurred (fig. 1). Regardless of which of the four different hemolymph combinations was administered, no significant

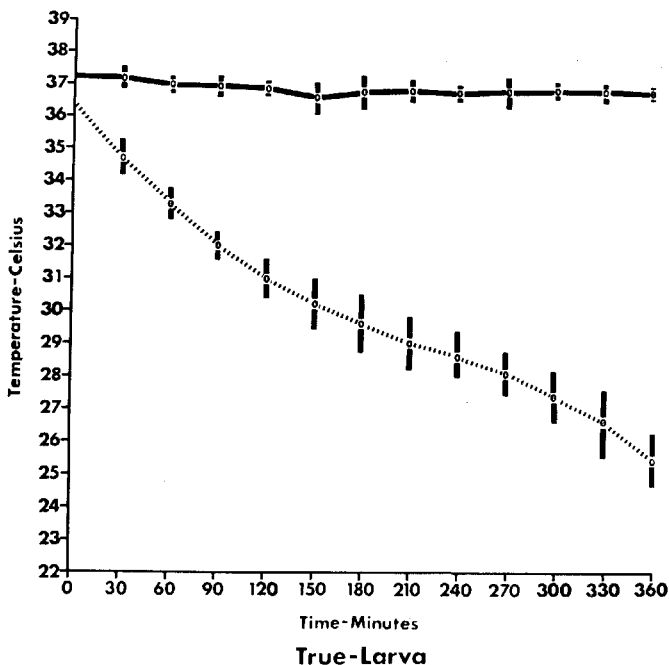


FIGURE 1. Temperature differences between control-grasshopper- and beetle-hemolymph-injected white rats. Curve drawn through experimental beetle-hemolymph data is dotted; curve through control data is solid. Fifty  $\mu$ l of hemolymph was injected intraperitoneally into rats in both instances. Grasshopper hemolymph was obtained from adults. Beetle hemolymph was obtained from fourth-instar larvae. Ten control and ten experimental animals were used to obtain these data. Standard deviation is indicated by length of bars.

temperature differences could be detected among the various combinations used on the rats. This observed decrease in temperature continued for six hours, after which monitoring was terminated. All hemolymph-injected animals died within twenty-four hours. During monitoring, there were no observable changes in rate of respiration or of heart beat.

After injection of beetle hemolymph, the hematocrit rose from 45 percent to about 70 percent (fig. 2). Increase in blood urea was dramatic, with urea increasing twofold within four hours after injection of beetle hemolymph (fig. 3). No

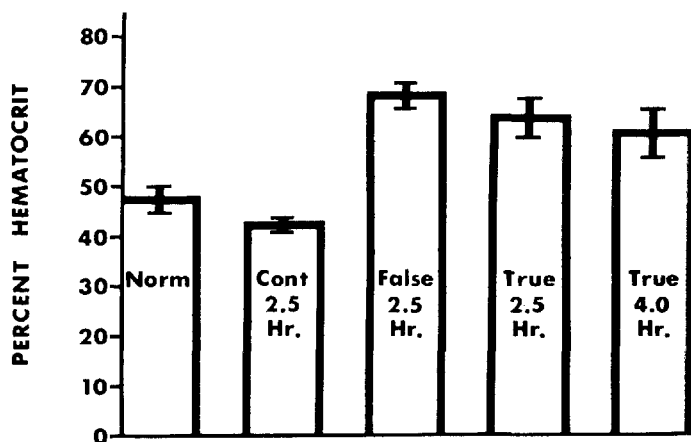


FIGURE 2. Comparison of hematocrit percentage changes. Control animals were injected intraperitoneally with  $50 \mu\text{l}$  of adult-grasshopper hemolymph. Other test animals were injected intraperitoneally with  $50 \mu\text{l}$  of true and of false adult beetle hemolymph. Ten animals were used to obtain each plot. A total of 50 animals was used for this figure. Standard deviation is indicated by length of bars.

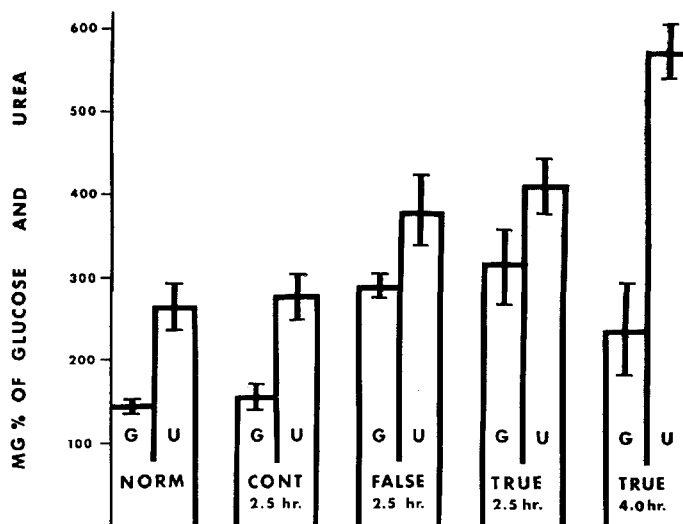


FIGURE 3. Comparison of changes in serum glucose and blood-urea levels. Control animals were injected intraperitoneally with  $50 \mu\text{l}$  of grasshopper hemolymph. Test animals were injected intraperitoneally with  $50 \mu\text{l}$  of true and of false adult beetle hemolymph. The same rats used to obtain data in figure 2 were used here. Standard deviation is indicated by length of bars.

significant differences were observed between hematocrit, blood urea, or serum glucose of controls injected with grasshopper hemolymph or of nontreated animals.

Serum transaminase and LDH enzyme activities were all elevated in the hemolymph-injected rats (fig. 4). Serum was collected from the rats four hours after administration of beetle hemolymph. Rats injected with beetle hemolymph developed an intraperitoneal effusion of 3–6 ml of fluid. Because of the abundance of this abdominal fluid, enzymatic activities of the effusate were also determined (fig. 4). Enzymatic activities of the enzymes monitored in the abdominal fluid

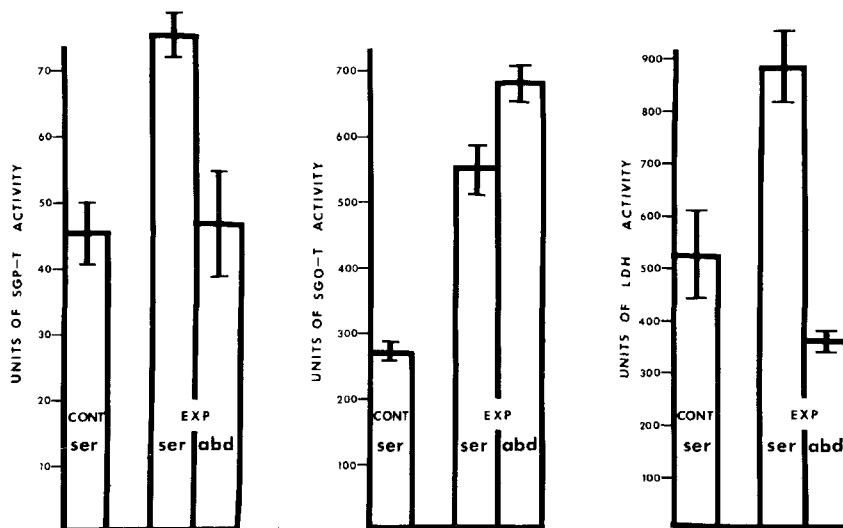


FIGURE 4. Comparison of changes in enzyme activities. Serum glutamic pyruvic transaminase (SGP-T), serum glutamic oxalacetic transaminase (SGO-T), and lactic acid dehydrogenase (LDH) activities are given in international units for the control serum and for the serum (ser) and abdominal fluid (abd) of experimental animals. Control animals were injected intraperitoneally with 50  $\mu$ l of adult-grasshopper hemolymph. Test animals were injected intraperitoneally with 50  $\mu$ l of true-adult-beetle hemolymph. Ten control and ten experimental animals were used to obtain these data. Standard deviation is indicated by length of bars.

were not consistently elevated in comparison to the levels found in the serum of beetle-hemolymph-injected and noninjected rats.

Rats injected with hemolymph had increases in serum potassium and inorganic phosphorus levels, whereas sodium and calcium levels were not significantly altered compared to control-rat values (fig. 5). Sodium levels were constant and similar between experimental and control animals, whereas potassium, calcium, and phosphorus levels were elevated in experimental animals (fig. 5).

#### DISCUSSION AND CONCLUSIONS

The overall effects on rats injected intraperitoneally with beetle hemolymph are suggestive of shock. More specifically, however, there appears to be cellular damage involving the blood system; this could be indicative of an anaphylactic shock. Changed concentrations of ions, urea, glucose, and enzymes could in part be explained by cell constituents leaking into the blood. Increased blood-enzyme activities are commonly used to indicate cellular damage. Beetle-hemolymph-

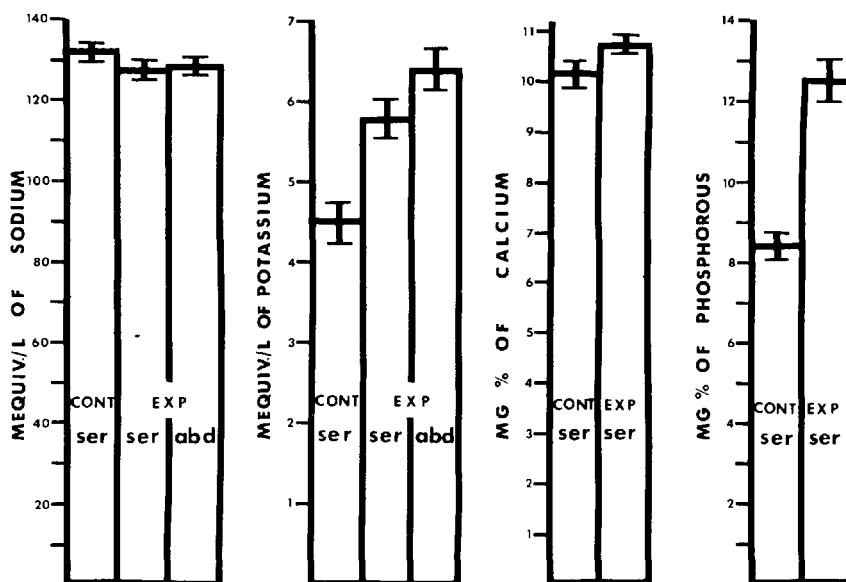


FIGURE 5. Comparison of changes in electrolyte levels, showing serum (ser) electrolytes as well as abdominal fluid (abd) sodium and potassium levels of experimental animals as compared to controls. Control animals were injected intraperitoneally with 50  $\mu$ l of adult-grasshopper hemolymph. Test animals were injected intraperitoneally with 50  $\mu$ l of true-adult-beetle hemolymph. Ten control and ten experimental animals were used to obtain these data. The same animals used to obtain data for figure 4 were used here. Standard deviation is indicated by length of bars.

injected rats produced large volumes (3–6 ml) of fluid in the peritoneal cavity. Microscopic examinations of this effusion fluid disclosed the presence of a small number of intact and hemolyzed erythrocytes. The increased hematocrit could be the result either of increased circulating cells or of a loss of the circulating fluid. Since the hematocrit changed dramatically in a short period of time (four hours), the loss of circulating fluid is the more plausible answer. Significant activities of LDH and transaminases were also found in the abdominal fluid.

The greatest perturbation initiated by beetle-hemolymph injections appears to involve the membrane integrity of the blood vessels in the rat's peritoneal cavity. The quantity of fluid found in the peritoneal cavity demonstrated that internal bleeding occurred. As would be expected from the data presented, the beetle-hemolymph-injected animals appeared to be in a state of shock.

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#### REFERENCES CITED

- Archibald, R. M. 1945. Colorimetric determination of urea. *J. Biol. Chem.* 157: 507–518.  
 Bett, I. M., and G. P. Fraser. 1959. A rapid micro-method for determining serum calcium. *Clin. Chim. Acta.* 4: 346–356.  
 Cabaud, P. G., and F. Wroblewski. 1958. Colorimetric measurements of lactic dehydrogenase activity of body fluids. *Amer. J. Clin. Path.* 30: 234–236.  
 Holiday, E. R., and J. R. K. Preedy. 1953. The precision of a direct-reading flame photometer for the determination of sodium and potassium in biological fluids. *Bio. Chem. J.* 55: 214–220.

- Hsaio, T. H., and G. Fraenkel.** 1969. Properties of leptinotarsin, a toxic hemolymph protein from the Colorado potato beetle. *Toxicon*. 7: 119-130.
- Raabo, E., and T. C. Terkildsen.** 1960. On the enzymatic determination of blood glucose. *Scand. J. Clin. and Lab. Invest.* 12: 402-407.
- Reitman, S., and S. Frankel.** 1957. Colorimetric method for the determination of serum transaminase activity. *Amer. J. Clin. Path.* 28: 56-63.
- Tausky, H. H., and E. Shore.** 1953. A microcolorimetric method for the determination of inorganic phosphorus. *J. Biol. Chem.* 202: 675-685.
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